The steady-state pharmacokinetics of nelfinavir in combination with tenofovir in HIV-infected patients

Guido Kruse1, Stefan Esser2, Hartmut Stocker3,4, Antje Breske1, Andrea Koerber2, Maija Kopperman2, Heidi Wicher2, Birgit Ross2, Christiane Moccklinghoff2, Andrew Hill6, Mark Becker7 and Michael Kurowski1,4*

1Therapia GmbH, Berlin, Germany
2University of Essen, Essen, Germany
3Auguste Viktoria Hospital, Berlin, Germany
4German HIV/AIDS Competence Network, Germany
5Roche, Grenzach, Germany
6University of Liverpool, Liverpool, UK
7Roche, Basel, Switzerland

*Corresponding author: Tel: +49 30 7903 3920; Fax: +49 30 7903 3922; E-mail: labor-kurowski@t-online.de

Background: The nucleotide analogue, tenofovir, has been shown to lower plasma atazanavir levels in pharmacokinetic trials, an interaction that may be partly reversed by the addition of ritonavir, whereas plasma tenofovir levels are themselves raised when the drug is combined with lopinavir/ritonavir.

Objective: To investigate the effect of tenofovir coadministration on the steady-state pharmacokinetics of nelfinavir in HIV-infected patients.

Methods: Eighteen patients received nelfinavir 1250 mg twice daily plus prescribed nucleoside reverse transcriptase inhibitors for at least 14 days, with pharmacokinetic measurements performed on day 15. Treatment with nelfinavir was continued for another 7 days with the addition of 300 mg tenofovir once daily. Pharmacokinetic measurements were repeated on day 22. Plasma samples were analysed by liquid chromatography–tandem mass spectrometry for nelfinavir, its primary metabolite, M8, and tenofovir. The parameters AUC0–12, C0, Cmax and Tmax were compared for nelfinavir with and without tenofovir by calculating geometric mean ratios (GMRs) of the pharmacokinetic parameters with associated 95% confidence intervals (95% CIs). Safety was assessed throughout the study.

Results: The addition of tenofovir to the nelfinavir-based regimen had no effect on the pharmacokinetics of nelfinavir. The GMR of the nelfinavir AUC0–12 values was 0.97 (95% CI: 0.80–1.17). There was a slight decrease in M8 metabolite (AUC0–12 ratio, 0.87; 95% CI: 0.68–1.11) but this was not significant. No serious adverse events occurred through the study period.

Conclusion: Nelfinavir does not require dose adjustment when coadministered with tenofovir and appears to be well-tolerated by HIV-infected patients.

Introduction

Several pharmacokinetic (PK) interactions have been described between the nucleotide analogue tenofovir disoproxil fumarate, the orally bioavailable prodrug of tenofovir, and other antiretroviral agents, including protease inhibitors (PIs) [1]. As tenofovir is eliminated predominantly by the renal route [2] and PIs are extensively metabolized in the liver, the mechanisms underlying these interactions are unclear. It has been suggested, however, that these interactions might occur at the drug absorption stage [3]. Recently, it was shown that tenofovir leads to a reduction in exposure to concomitantly administered atazanavir, which is consistent with a significant interaction between tenofovir and this new PI [3]. Conversely, plasma tenofovir levels were themselves raised when the nucleotide analogue was combined with ritonavir-boosted lopinavir [4]. The unpredictability of these tenofovir-associated interactions, together with the popularity of tenofovir as an antiretroviral agent, necessitates careful examination of the steady-state pharmacokinetics of antiretroviral agents that might be coadministered with it.

Nelfinavir-based highly active antiretroviral therapy (HAART) has demonstrated potent and durable virological control of HIV for at least 4 years [5–7]. Data from large, randomized clinical trials show nelfinavir to have comparable efficacy with more recently developed PIs, such as atazanavir and ritonavir-boosted fosamprenavir [8–11], as well as a good tolerability and safety.
profile, which is characterized by low rates of toxicity and treatment-related discontinuations [7]. In addition, the absence of mutations at failure or the selection of the unique D30N resistance pathway by nelfinavir-based regimens may allow the subsequent successful use of a boosted PI [12–15]. Furthermore, nelfinavir remains the only commercially available PI that is not routinely combined with boosting doses of ritonavir, and as the interaction with tenofovir appears more pronounced with unboosted atazanavir, investigation of the effect of tenofovir on nelfinavir pharmacokinetics was warranted.

Methods

Study population
HIV-infected patients over the age of 18 were eligible for this study if they were taking a nelfinavir-containing regimen, with nelfinavir dosed at 1250 mg twice daily for at least 14 days prior to study entry, had no significant clinical and laboratory findings during pre-study screening and were able to provide written consent to comply with study requirements. Patients were excluded if they had any history or clinical findings that might interfere with the pharmacokinetics of the study drugs, if they had received any investigational drug within 3 months prior to the start of the study, if they had a recent (<3 years) history of active substance abuse or were intolerant to any of the study drugs. Patients were also excluded if they had received any co-medication judged likely to interfere with nelfinavir or tenofovir (such as known inducers/inhibitors of the CYP3A4 enzyme) in the 2 weeks prior to study entry.

The study was conducted in accordance with the principles of the Declaration of Helsinki and adhered to the recent versions of both the German and European Guidelines for Good Clinical Practice.

Study design
This prospective Phase I trial was a single-centre, open-label, multiple-dose, single-group study. The study design is shown in Figure 1.

After a pre-study phase of at least 14 days with orally administered nelfinavir 1250 mg twice daily (Viracept® 5×250 mg tablets) + two nucleoside reverse transcriptase inhibitors (NRTIs), PK measurements were performed at day 15. The patients then continued taking this regimen for a further 7 days (investigational phase) whilst also receiving oral tenofovir 300 mg once daily (Viread® 1×300 mg tablets). On day 22, PK measurements were repeated and on day 23 pre-study dosing resumed if deemed appropriate by the investigator. A follow-up examination was conducted within 14 days of this second PK assessment.

The course of the study period was identical for each patient enrolled. A pre-study examination was carried out 2–14 days prior to enrolment into the investigational phase. Medical history was recorded and a physical examination was conducted. Vital signs and body temperature were measured, and blood and urine sampled for routine laboratory parameters.

Safety was assessed throughout the study. Subjects were asked whether they had experienced any adverse events (AEs) on days 15 and 22 and at the follow-up visit. Repeat laboratory tests were carried out on day 22 and during the follow-up visit; physical examination was repeated at follow-up.

Blood sample collection and preparation
Patients were required to keep a journal recording their compliance to the study regimen and the two doses of

Figure 1. Study design

![Study design diagram](image-url)
study drugs prior to PK sampling were observed. On days 15 and 22, patients were admitted to the study unit for PK sampling. On both of these occasions blood samples were collected for the determination of plasma concentrations of nelfinavir, the nelfinavir metabolite M8 (nelfinavir hydroxy-t-butylamide) and, if appropriate, tenofovir, using identical sampling schedules on days 15 and 22. Blood samples (3 ml) were drawn 15 min prior to dosing (time 0), then at 1, 2, 3, 4, 6, 8, 10 and 12 h post-dose. All samples were centrifuged within 1 h of collection and plasma transferred immediately to a −20°C freezer for storage until analysis.

Analytical methods
The analytical method for the determination of plasma concentrations of the tenofovir metabolite 9-[2-(R)- (phosphomethoxy)propyl]adenine (PMPA) has recently been developed and fully validated by the authors. In brief, 0.1 ml of serum was extracted with acetonitrile (0.5 ml) and centrifuged. The supernatant was removed and reduced under vacuum to approximately 0.05 ml, to which 0.1 ml of the aqueous chromatographic eluent was added, and the resulting solutions stored at −25°C prior to drug analysis. Thawed samples were chromatographed on a Luna C18 HPLC column (Phenomenex, Torrance, CA, USA) using gradient elution. The aqueous mobile phase was 5 mM ammonium acetate buffered to pH 7.5 with ammonia, and the organic phase was acetonitrile containing 0.1% formic acid. An API 3000 mass spectrometer (Applied Biosystems, Streetsville, ON, Canada) equipped with an ESI interface and run with Analyst 1.2 software was used for peak detection, integration and quantification. Quantification of PMPA was achieved by reference to the internal standard 2-chloroadenosine (Sigma, St Louis, MO, USA).

Concentrations of nelfinavir and M8 were quantified by liquid chromatography–tandem mass spectrometry (LC/MS/MS) according to a validated method [16]. During the study, all subjects who met study criteria had a physician-selected NRTI backbone consisting of two of the following: lamivudine (n=11), zidovudine (n=7), stavudine (n=6), didanosine (n=1) and abacavir (n=1). Baseline antiretroviral medication was maintained unchanged throughout the study. The only other concomitant medication taken by more than two patients at any time during the study was pravastatin (n=3).

Pharmacokinetic analyses
Data are presented for all 13 patients who met study criteria. Geometric mean concentration–time profiles for nelfinavir and M8 are shown in Figures 2 and 3, respectively. During the first PK sampling period (study day 15, without tenofovir), prior to dosing with nelfinavir 1250 mg, the geometric mean C0 was 1338 ng/ml. Concentrations increased over the next few hours, reaching a geometric mean Cmax of 3753 ng/ml 4 h post-dose, and time to Cmax (Tmax).

These PK parameters were also determined for tenofovir on day 22. All pharmacokinetic parameters were analysed descriptively including arithmetic mean, standard deviation, geometric mean, minimum, maximum and median. The nelfinavir concentration–time profiles at day 15 and day 22 were compared by calculation of geometric mean ratios (GMRs) for the log transformed AUC0–12 and Cmax including 95% confidence intervals (95% CI).

Results
Patient demographics
Nineteen patients undergoing long-term nelfinavir-containing treatment for HIV infection underwent pre-study screening. Of these 19, six failed to complete the study according to the protocol: one refused to continue, two continued to take nelfinavir according to a three times daily dosing schedule, two were found to have taken nevirapine and one had taken simvastatin, which, as substrates of CYP3A4, may interact pharmacokinetically with nelfinavir. Baseline demographics for the 13 patients who met study criteria are presented in Table 1.

During the study, all subjects who met study criteria had undetectable viral load (<50 copies/ml) at baseline. Of these 19, six failed to complete the study according to the protocol: one refused to continue, two continued to take nelfinavir according to a three times daily dosing schedule, two were found to have taken nevirapine and one had taken simvastatin, which, as substrates of CYP3A4, may interact pharmacokinetically with nelfinavir. Baseline demographics for the 13 patients who met study criteria are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male/female, n (%)+</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
</tr>
<tr>
<td>CD4 cell count, cells/mm³</td>
</tr>
<tr>
<td>HIV viral load, copies/ml</td>
</tr>
</tbody>
</table>

*Unless otherwise specified. †Baseline CD4 cell count and viral load only available for 12 patients. ‡Mean viral load not available as seven out of 12 patients had undetectable viral load (<50 copies/ml) at baseline.
**Figure 2.** Geometric mean 12-h nelfinavir plasma concentration–time profiles for nelfinavir 1250 mg twice daily, administered with (day 22) and without (day 15) tenofovir 300 mg once daily (n=13)

![Graph showing geometric mean 12-h nelfinavir plasma concentration–time profiles](image)

NFV, nelfinavir; TDF, tenofovir.

**Figure 3.** Geometric mean 12-h M8 plasma concentration–time profiles for nelfinavir 1250 mg twice daily, administered with (day 22) and without (day 15) tenofovir 300 mg once daily (n=13)

![Graph showing geometric mean 12-h M8 plasma concentration–time profiles](image)

TDF, tenofovir.
after 4 h. Subsequently, levels of nelfinavir declined over 8 h and measured 1414 ng/ml at the end of the 12-h dosing interval. PK profiles of the M8 metabolite exhibited a similar time course. Geometric mean C₀ was 370 ng/ml, representing 27.2% of the corresponding nelfinavir concentration, with a Cₘₐₓ of 1261 ng/ml observed after 5 h. During the elimination phase, the M8 concentrations decreased to 436 ng/ml after 12 h. For both compounds, similar profiles were obtained on day 22 following 7 days of tenofovir coadministered with the same dose of nelfinavir. Nelfinavir concentrations started at 1390 ng/ml, reached maximum values of 3729 ng/ml after 3 h and declined to 1279 ng/ml after 12 h. The respective geometric mean concentration of the M8 metabolite was found to be 356 ng/ml at time 0, reaching a Cₘₐₓ of 1076 ng/ml after 3 h before falling to a final concentration of 390 ng/ml after 12 h. Nelfinavir’s geometric mean AUC₀–12 for the observed 12-h dosing interval was found to be 28 830 ng ·h/ml without tenofovir and 27 700 ng ·h/ml with tenofovir, respectively. The corresponding values for the M8 metabolite were 8392 ng ·h/ml and 7301 ng ·h/ml, in the absence and presence of tenofovir, respectively.

Safety
There were no serious AEs and no deaths during the study. Amongst the 18 patients who completed the study, there were a total of 10 AE episodes reported during the tenofovir coadministration (investigational) phase. All were mild to moderate in nature and none resulted in discontinuation. Of these, four AE episodes, rated as mild, were deemed possibly related to study medication: nausea (2 episodes), diarrhoea (1) and dysgeusia (1).

Discussion
The use of combination antiretroviral therapy has led to marked reductions in the morbidity and mortality associated with HIV/AIDS [17]. However, inherent in the use of these necessarily complex antiretroviral combinations is the risk of drug–drug interactions and their subsequent effect on optimal drug exposure. While interactions between concomitantly administered

| Table 2. Nelfinavir and M8 PK parameters [expressed as geometric means and GMRs* (with 95% CIs)] for nelfinavir 1250 mg twice daily administered with (day 22) and without (day 15) tenofovir 300 mg once daily (n=13) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                 | Without TDF day 15              | With TDF day 22                 | GMR day 22/day 15                |
| NFV                             |                                 |                                 |                                 |
| AUC₀–12 , ng·h/ml               | 28 830 (21 300–39 010)          | 27 700 (20 530–37 360)          | 0.97 (0.80–1.17)                |
| Cₘₐₓ, ng/ml                     | 3753 (2892–4872)                | 3729 (2841–4895)                | 0.99 (0.80–1.24)                |
| C₀, ng/ml                       | 1358 (768–2402)                 | 1390 (882–2189)                 | 1.02 (0.66–1.58)                |
| Tmax, h                         | 4 (3–6)                         | 3 (1–8)                         | 0.65 (0.37–1.15)                |
| M8                              |                                 |                                 |                                 |
| AUC₀–12 , ng·h/ml               | 8392 (4808–14 650)              | 7301 (4432–12 030)              | 0.87 (0.68–1.11)                |
| Cₘₐₓ, ng/ml                     | 1261 (718–2214)                 | 1076 (620–1864)                 | 0.85 (0.68–1.11)                |
| C₀, ng/ml                       | 370 (161–850)                   | 356 (198–641)                   | 0.96 (0.65–1.11)                |
| Tmax, h                         | 5 (4–7)                         | 3 (1–10)                        | 0.63 (0.36–1.12)                |

*The geometric mean ratio (GMR) compares PK values across the two study days. A GMR of 1 means no change in drug levels; a GMR of 2 means a 100% rise in drug levels. If the confidence intervals (CIs) of the GMRs include 1 then the change is not statistically significant. NFV, nelfinavir; TDF, tenofovir.

Table 3. Tenofovir PK parameters [expressed as geometric means (with 95% CIs)] for tenofovir 300 mg once daily administered with nelfinavir 1250 mg twice daily (n=15)

<table>
<thead>
<tr>
<th>Tenofovir</th>
<th>With nelfinavir day 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC₀–12 , ng·h/ml</td>
<td>2899 (1965–4277)</td>
</tr>
<tr>
<td>Cₘₐₓ, ng/ml</td>
<td>499 (323–770)</td>
</tr>
<tr>
<td>C₀, ng/ml</td>
<td>111 (85–144)</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>2 (1–2)</td>
</tr>
</tbody>
</table>

For PMPA was found to be 111 ng/ml. A Cₘₐₓ of 499 ng/ml was observed after 2 h, with a final concentration of 147 ng/ml observed 12 h post-dose.
antiretrovirals can sometimes prove beneficial, as with the use of low-dose ritonavir to boost drug plasma levels of a coadministered PI, negative interactions may reduce drug levels to a point where they are no longer sufficient and virological control is lost. In order to achieve optimal regimens it is therefore important that any interactions are fully understood. Reports of the potential for drug–drug interactions with tenofovir have spurred PK interaction studies of existing licensed antiretrovirals with this agent.

The pharmacokinetics of nelfinavir after multiple dosing are different from those after single-dose administration [6,18]. Following multiple-dose administration, nelfinavir has been found to induce its own metabolism. Nelfinavir levels reach steady state after approximately 1 week and have been found to be slightly lower than after single-dose administration [19]. The pharmacokinetics after single-dose administration have not been helpful in predicting steady-state pharmacokinetics of nelfinavir. It is therefore necessary to evaluate drug interactions after steady state has been reached for nelfinavir. Given that the elimination half-life of tenofovir is 17 h [20], it was felt that an investigational phase of 7 days — the equivalent of 10 half-lives — should be sufficient to ensure steady-state conditions for this drug.

The aim of the present study was to investigate the effect of tenofovir (300 mg once daily) coadministration on the steady-state pharmacokinetics of nelfinavir (1250 mg twice daily) in HIV-infected patients. The results showed that the addition of tenofovir to a nelfinavir-based treatment regimen had no effect on the pharmacokinetic parameters of nelfinavir. The GMR of nelfinavir AUC values comparing exposure in the absence and presence of tenofovir was 0.97 (95% CI: 0.80–1.17). Similarly there was no difference in C₀ for nelfinavir regardless of whether tenofovir was coadministered or not (GMR 1.02; 95% CI: 0.66–1.58). A slight decrease in exposure to the M8 metabolite was noted, but this was not statistically significant (AUC GMR 0.87; 95% CI: 0.68–1.11). Again, there was no significant difference in the M8 C₀ values (C₀ GMR 0.96; 95% CI: 0.65–1.11).

This study was not designed to assess the effect of nelfinavir on tenofovir, so tenofovir parameters can only be compared with historical controls, which has well-described limitations. In this study, the mean pharmacokinetic results for tenofovir were slightly greater than previously reported [20]. This study also demonstrated that the combination of nelfinavir and tenofovir was not associated with any new safety or tolerability issues, at least in the short term.
The widespread use of nelfinavir, particularly in challenging patient populations such as pregnant women and hepatitis C-coinfected patients, plus the popularity of tenofovir, suggest that this may be a frequently used combination. This study shows that nelfinavir 1250 mg twice daily does not require dose adjustment when coadministered with tenofovir 300 mg once daily and that this combination can be regarded as a well-tolerated treatment for HIV-infected patients.

Acknowledgements

The authors would like to express their gratitude to all the patients, investigators and study centre staff that participated in the trial, as well as the Roche study personnel who worked on the study. Supported by Roche.

References